

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
14 June 2001 (14.06.2001)

PCT

(10) International Publication Number  
**WO 01/41778 A1**

(51) International Patent Classification<sup>7</sup>: **A61K 35/78**

(21) International Application Number: **PCT/KR00/01420**

(22) International Filing Date: **7 December 2000 (07.12.2000)**

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:  
**1999/55670 8 December 1999 (08.12.1999) KR**

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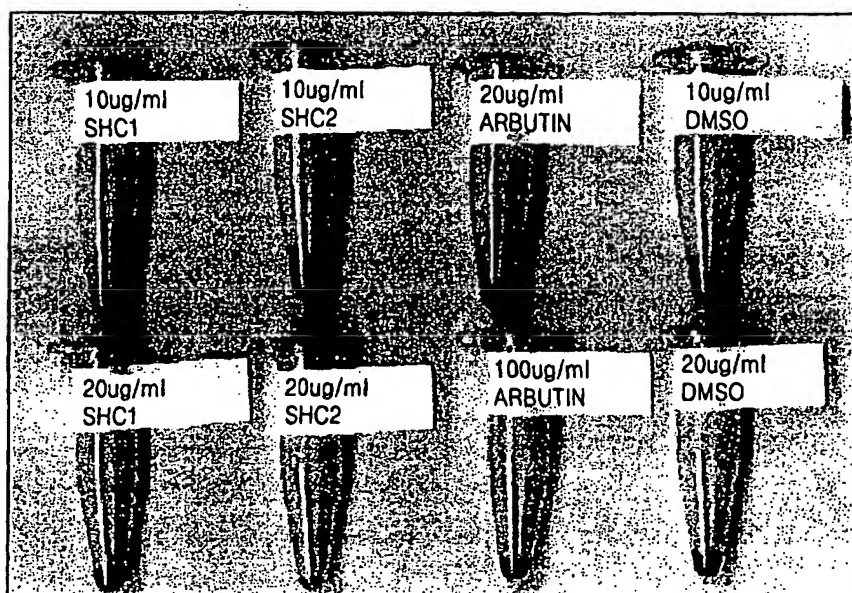
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(81) Designated States (*national*): **AE, AG, AL, AM, AT, AU,**  
**AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,**  
**DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,**  
**HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS,**  
**LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,**  
**NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,**  
**TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.**

(84) Designated States (*regional*): **ARIPO patent (GH, GM,**  
**KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian**

[Continued on next page]

(54) Title: **MELANIN SYNTHESIS INHIBITION COMPOUND AND COMPOSITION CONTAINING THE SAME**



(57) Abstract: The present invention relates to melanin synthesis inhibition compound containing gomisin N or  $\gamma$ -schizandrin and composition containing the same. The composition of the present invention has a superb melanin synthesis inhibition effect without showing cytotoxicity. Also the present invention relates to a schizandra extract that has a excellent melanin synthesis inhibition effect without showing cytotoxicity.



patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**Published:**

— *With international search report.*

## **MELANIN SYNTHESIS INHIBITION COMPOUND AND COMPOSITION CONTAINING THE SAME**

### **FIELD OF INVENTION**

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The present invention relates to a compound having melanin synthesis inhibition effect and composition containing the same.

### **BACKGROUND OF THE INVENTION**

10

When skin is exposed to sunlight, melanin pigment is synthesized to prevent skin from ultraviolet rays. The synthesized melanin moves up to the dermal surface to darken skin.

Melanin has the structure of phenol ring that exists widely in nature and is  
15 a complex of black pigment and protein. Melanin is generally divided physicochemically into three categories, yellow-redish brown pheomelanin, brown-black eumelanin, and trichochrome that has a similar characteristic. It is reported that tyrosinase (EC 1.14.18.1) plays an active role in the biosynthesis of all three types of melanin. The human skin tone is determined by the type and  
20 the amount of the melanin pigment. Excessive production of melanin can induce the formation of cholasma and freckles and promotes the skin aging. Also it can induce the skin cancer. Melanin is known for degrading the quality of vegetables, fruits and fish in food science.

Melanin synthesis inhibitors are applied as topical ointment, skin whitener and food coloration inhibitor in pharmaceuticals, cosmetics and food industry and the demand is expanding in recent years.

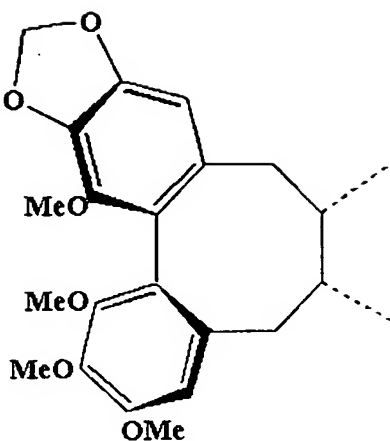
The research on the melanin synthesis inhibitors has been mainly concentrated on the development of tyrosinase inhibitor. As a result, a variety of skin whiteners have been developed and currently used. Many associated problems, however, have been recognized.

4-Hydroxylanizole and hydroquinone are locally applied in the treatment of excessive pigmentation such as cholasma, freckles, blotch and excessive pigmentation during pregnancy. Even though these compounds have strong inhibitory activities of melanin production, they are allowed to be used in only a few countries since they exhibit the side-effects including the alteration and necrosis of pigment cells and the damage of cell function. Also Kojic acid [5-hydroxy-2-(hydroxymethyl- $\gamma$ -pyrone)] and albutin (hydroquinone- $\beta$ -D-glucopyranoside) along with ascorbic acid are used as the main active ingredients of food coloration inhibitor, cosmetic or pharmaceutical whitener. The use of these inhibitors has associated problems, however, including low inhibitory activities and their own instabilities. The color of these compounds themselves sometimes changes during use.

Korean patent 228741 has shown the solid food containing the schizandra extract that has a tooth whitening effect and prevents tooth decay since the extract exhibits the tyrosinase and glucosyltransferase inhibition activity.

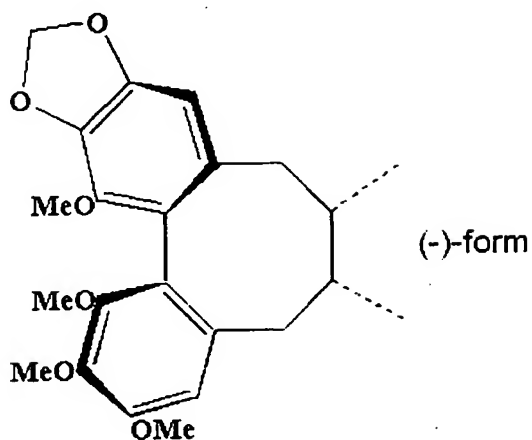
Gomisin N and  $\gamma$ -schizandrin are stereoisomers each other with the

following structures.



Gomisin N

5



$\gamma$ -schizandrin

It is reported that gomisin N inhibits the activity of cholesterol  
10 acyltransferase (Planta med. 1999, 65: 74-76), and also inhibits the activity of  
chitinase II that is a cell wall synthesizing enzyme of fungi (Journal of the  
Pharmaceutical Society of Korea, 1995, 43(4): 509-515).

The physiological activity of  $\gamma$ -schizandrin is not known at present.

## SUMMARY OF THE INVENTION

5 It is an object of the invention to provide the schizandra extract that has a melanin inhibition activity.

Another object of the invention is to provide a melanin synthesis inhibition compound.

Another object of the invention is to provide a melanin synthesis inhibition  
10 composition.

## BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a graph representing the tyrosinase activity inhibition effect of  
15 gomisin N and  $\gamma$ -schizandrin.

Figure 2 is a graph representing the antioxidant activity of gomisin N and  $\gamma$ -schizandrin.

Figure 3 is a photograph showing the inhibition effect of gomisin N and  $\gamma$ -schizandrin on aspergillin synthesis.

20 Figure 4 is a photograph showing the inhibition effect of gomisin N and  $\gamma$ -schizandrin on melanin synthesis through *in vitro* cell culture.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to a melanin synthesis inhibition compound containing the schizandra extract. The schizandra extract of the present invention preferably contains gomisin N or  $\gamma$ -schizandrin.

The present invention also relates to a melanin synthesis inhibition composition containing gomisin N or  $\gamma$ -schizandrin.

The present inventors have found that Gomisin N and  $\gamma$ -schizandrin have negligible cytotoxicity but have an excellent whitening effect.

Gomisin N and  $\gamma$ -schizandrin have a considerably lower tyrosinase inhibition activity than other conventional compounds having whitening effect, for instance kojic acid or albutin. From this fact, we can deduce that the melanin synthesis inhibition activity of gomisin N and  $\gamma$ -schizandrin does not originate from the tyrosinase inhibition mechanism. Also from the fact that gomisin N and  $\gamma$ -schizandrin have a low antioxidant activity, it can also be known that Gomisin N and  $\gamma$ -schizandrin don't inhibit the oxidation process in the melanin synthesis starting from tyrosine. Also, gomisin N and  $\gamma$ -schizandrin selectively inhibit the pigment synthesis without showing cytotoxicity.

Gomisin N and  $\gamma$ -schizandrin can be extracted from biological organisms or prepared by synthesis or other known methods in the field. For instance, gomisin N or  $\gamma$ -schizandrin can be extracted from schizandra.

The composition containing gomisin N or  $\gamma$ -schizandrin according to the

present invention can be used as a melanin synthesis inhibitor and can be utilized as an cosmetic, pharmaceutical whitener or food coloration inhibitor. The specific contents in the composition according to the present invention can be easily determined by the specialists having conventional knowledge in the field.

5           The method to determine the activity of the whitener is as follows.

#### Determination of degree of tyrosinase activity inhibition

To determine the degree of tyrosinase activity inhibition, 0.1 mg/ml of tyrosine (Sigma, USA) was added in 1.5 ml of 50 mM phosphate buffer solution (pH 6.8) in a 35 °C preset waterbath, and 110 unit/ml of tyrosinase (EC 1.14.18.1; 10 4,400unit/mg powder, Sigma) was further added. In this mixture, serially diluted samples were added and reacted at 37 °C for 10 min. The absorbance of the samples,  $S_{Abs}$  was measured at 475 nm.  $B_{Abs}$  is the absorbance measured by adding distilled water instead of the enzyme solution, and  $C_{Abs}$  is the absorbance 15 measured by adding distilled water instead of the extract samples. The following formula was used to determine inhibition percentage.

$$\text{Inhibition (\%)} = \left[ 1 - \left( \frac{S_{Abs} - B_{Abs}}{C_{Abs}} \right) \right] \times 100$$

#### Determination of antioxidant activity

The antioxidant activity of a compound was determined as follows. Each 20 compound (0.1 mg) was dissolved in 0.1 ml methanol. In this solution, 5 ml ethanol containing 58 mg of linoleic acid and 5 ml of 0.2 M phosphate buffer



solution (pH 7.0) were added. Distilled water was also added to make the total volume to be 12 ml, and the mixture was incubated in a 40 °C incubator. After 24 hours, the absorbance was measured at 500 nm by mixing 7 ml of 75 % ethanol, 0.1 ml of reaction mixture, 150 µl of 0.02 M FeCl<sub>2</sub> solution and 150 µl of 30% ammonium thiocyanate and reacting for 3 minutes.

Determination of the melanin synthesis inhibition activity of *Streptomyces bikiniensis*

*S.bikiniensis* KCTC 9172 was inoculated into Papavizas VDYA slant medium at pH 7.2 containing 200 ml of V-8 juice, 2 g of glucose, 2 g of yeast extract, 1 g of CaCO<sub>3</sub>, 2g of bacto agar and 800 ml of distilled water. On the slant culture surface cultivated for 2 days at 28 °C, 2 ml of distilled water was added. The spores on the aerial mycelium were gathered with a platinum loop to prepare spore suspension.

In tyrosine agar medium containing 1.5 % glucose, 0.5 % L-tyrosine, 0.1 % L-asparagine, 0.05 % K<sub>2</sub>HPO<sub>4</sub>, 0.05 % MgSO<sub>4</sub> ·7H<sub>2</sub>O, 0.05 % NaCl, 0.001 % FeSO<sub>4</sub> ·7H<sub>2</sub>O and 2 % bacto-agar, 0.2 % of bacto-yeast extract was added to prepare solid medium. The prepared solid medium was sterilized to prepare plate culture in petri-dishes. In each agar plate, 0.4 ml of *S.bikiniensis* spore suspension was homogeneously spread. A 8 mm paper disk containing 50 µl of sample solution was placed on the plate and cultured for 48 hours at 28 °C.

The diameter of the produced zone due to melanin production inhibition was determined on the rear side of the plate.  $\rho$ -Hydroxyanizole was used as a control.

5        Determination of melanin synthesis inhibition activity of *Aspergillus niger* spores

*A. niger* KCTC 2118 was cultivated in the potato dextrose agar medium (20 % potato, 2 % dextrose, 1.5 % agar; PDA medium) for 6 days at 37 °C. Tween 80 solution (10 ml, 0.01 %) was added to the surface of the plate to gather  
10 the spores formed on the mycelium and the spore suspension was obtained. After adding the spore suspension into PDA medium at 5 %, 1 ml of the medium was solidified in each of the 24 wells. The sample was dissolved in 50  $\mu$ l of ethanol and added on the surface of each well plate. The plate was cultivated for 2 days at 37 °C to observe the pigment synthesis from the spores. The  
15 number of the spores in 1 ml spore suspension was controlled to contain  $1.5 \times 10^5$  CFU.  $\rho$ -Hydroxyanizole was used as a control.

Determination of melanin synthesis inhibiting activity of melanoma cells

After suspending B16 mouse melanoma cells at  $5 \times 10^3$  cells/ml in DMEM  
20 medium (Dulbescco's Modified Eagle's Medium, Gibco BRL) supplemented with 10% FCS (fetal calf serum), 5 ml of the cell suspension was added in a cell culture flask (Falcon, Becton dickinson). The samples were added at different

concentrations and cultivated at 37 °C under the condition of 5% CO<sub>2</sub> - 95% air.

After 4 days of cultivation, the cells adhering onto the bottom of the flask were washed with phosphate buffered saline (PBS) solution. Cells were detached with 0.05 % trypsin and 0.53 mM EDTA solution, and centrifuged for 10 min at 5 1500 rpm. Cells were collected in the tube, and the cell number was counted. The number of cells in each tube was controlled to be equal. The color of the cells collected after centrifugation was compared with that of control group visually.

Also, the cells, obtained by the above centrifugation process, were treated 10 with 5 % trichloroacetate (TCA), stirred, and centrifuged to obtain the melanin precipitation. Precipitated melanin was washed with PBS solution. In the washed melanin, 1ml of 1N sodium hydroxide solution was added and boiled for 10 min to dissolve melanin. The amount of produced melanin per unit cells (10<sup>6</sup> cells) was represented as the absorbance value at 400 nm by measuring the 15 absorbance using a spectrophotometer. The relative amount of the produced melanin to that of control was considered as the percent inhibition (%) to calculate IC<sub>50</sub>.

The invention will be further illustrated by the following examples, but not limited to the examples given.

20

#### Example 1. Separation of melanin synthesis inhibition compound

After adding 10 L of methanol in 500 g of crushed schizandra, the mixture was incubated with shaking at room temperature for 48 hours and extracted twice.

After extraction, the mixture was filtered and concentrated at 50 °C at reduced pressure to obtain 298 g of the concentrated extract. Two liters of the mixture of hexane:water:methanol = 10:9:1 (V/V/V) was added into this extract to obtain the fractions of hexane and water layers. The extract in the hexane layer (21.4 g) exhibiting the melanin synthesis inhibition activity of *A. niger* spores was separated by using silica gel column chromatography (solvent system: benzene:acetone, 98:2→3:7 (V/V)) to obtain 12 fractions each having 200 ml of volume. The melanin synthesis inhibition activity in melanoma cells was determined with the fraction 2~5 showing melanin synthesis inhibition activity of *A. niger* spores. The results show that the fraction 3 has a melanin synthesis inhibition activity without showing the reduction in cell number as low as at 10 µg/ml concentration. Reverse phase column chromatography (70% methanol →100% methanol) was performed with the fraction 3 (3.1 g). Total 25 fractions each having 50 ml of volume were collected. The fraction 14 which showed the strongest activity was developed with preliminary run of thin layer chromatography (TLC, hexane:ethyl acetate = 6:1), and the band exhibiting melanin synthesis inhibition activity of *A. niger* spores was collected. Preliminary HPLC (column: Vydac ODS, 5 µm, 250×10 mm I.D, flow rate: 2 ml/min, UV detector: 22 nm, solvent: 70% acetonitrile) was performed with the collected solution of active band. Two compounds were separated and named SHC1 and SHC2. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were obtained, and the analysis result of these final two compounds is shown in Table 1.

Table 1

|                      | SHC1                                |  | SHC2                              |                                      |
|----------------------|-------------------------------------|--|-----------------------------------|--------------------------------------|
|                      | $\delta$ $^1\text{H}(\text{ppm})^a$ | $\delta$ $^{13}\text{C}(\text{ppm})^b$ | $\delta$ $^1\text{H}(\text{ppm})$ | $\delta$ $^{13}\text{C}(\text{ppm})$ |
| 1                    |                                     | 151.5                                  |                                   | 152.8                                |
| 2                    |                                     | 140.0                                  |                                   | 139.7                                |
| 3                    |                                     | 151.5                                  |                                   | 151.4                                |
| 4                    | 6.56                                | 110.6                                  | 6.55                              | 107.4                                |
| 5                    |                                     | 134.0                                  |                                   | 132.5                                |
| 6                    | 2.55                                | 39.0                                   | 2.47                              | 38.8                                 |
| 7                    | 1.78~1.91                           | 33.5                                   | 1.81~1.91                         | 33.8                                 |
| 8                    | 1.78~1.91                           | 40.6                                   | 1.81~1.91                         | 40.7                                 |
| 9                    | 2.20~2.27                           | 35.4                                   | 2.28~2.35                         | 35.4                                 |
| 10                   |                                     | 137.7                                  |                                   | 139.4                                |
| 11                   | 6.48                                | 102.8                                  | 6.49                              | 105.9                                |
| 12                   |                                     | 148.6                                  |                                   | 147.6                                |
| 13                   |                                     | 134.5                                  |                                   | 134.8                                |
| 14                   |                                     | 141.0                                  |                                   | 141.2                                |
| 15                   |                                     | 121.3                                  |                                   | 122.2                                |
| 16                   |                                     | 123.2                                  |                                   | 122.4                                |
| -CH <sub>3</sub>     | 0.99                                | 21.4                                   | 1.01                              | 21.9                                 |
|                      | 0.75                                | 12.7                                   | 0.75                              | 12.3                                 |
| -OCH <sub>3</sub>    | 3.55                                | 55.8                                   | 3.54                              | 55.8                                 |
|                      | 3.82                                | 59.5                                   | 3.83                              | 59.5                                 |
|                      | 3.89                                | 60.9                                   | 3.89                              | 60.9                                 |
|                      | 3.90                                | 60.4                                   | 3.90                              | 60.5                                 |
| -OCH <sub>2</sub> O- | 5.94                                | 100.6                                  | 5.96                              | 100.7                                |

<sup>a</sup>(CDCl<sub>3</sub>, 300MHz). <sup>b</sup>(CDCl<sub>3</sub>, 75MHz).

SHC 1 and SHC2 are both colorless oils, and have the same molecular  
 5 weight of 400 identified by the m/z value from EI-MS. From these results, SHC1

and SHC2 are considered to be stereoisomers with the molecular formula of  $C_{23}H_{28}O_6$ . SHC1 and SHC2 were assumed as gomisin N and  $\gamma$ -schizandrin, respectively. We have confirmed that SHC1 and SHC2 were indeed gomisin N and  $\gamma$ -schizandrin, respectively, by comparing the HPLC analysis result of the standard forms of gomisin N and  $\gamma$ -schizandrin and the literature values of physicochemical properties and NMR data of them.

#### Example 2. Determination of tyrosinase inhibition activity

Gomisin N and  $\gamma$ -schizandrin obtained from Example 1 were 1/2 serially diluted from 200 ppm to determine tyrosinase inhibition activity. The inhibition result was compared with the activities of positive controls of kojic acid, albutin and  $p$ -hydroxyanizole. The result is shown in Figure 1. In this figure, the following representations are used; ▲: kojic acid, ■: albutin, ●:  $p$ -hydroxyanizole, ○:  $\gamma$ -schizandrin, □: gomisin N. Kojic acid exhibited higher than 50 % inhibition at 10 ppm, albutin at 25 ppm and  $p$ -hydroxyanizole at 50 ppm. Gomisin N and  $\gamma$ -schizandrin, however, showed only weak activities even at higher than 200 ppm.

#### Example 3. Estimation of antioxidant activity

One hundred micrograms each of gomisin N and  $\gamma$ -schizandrin obtained from Example 1 were added in the reactant solution containing linoleic acid, respectively. The antioxidant activity was determined after 24 hours. Ascorbic

acid,  $\alpha$ -tocopherol and  $p$ -hydroxyanizole were used as controls. The result is shown in Figure 2. In this figure, the following representations are used; A: ascorbic acid, B:  $\alpha$ -tocopherol, C:  $p$ -hydroxyanizole, D: gomisin N, E:  $\gamma$ -schizandrin. The result shows that ascorbic acid has 72 % of the antioxidant activity after 24 hours.  $\alpha$ -Tocopherol and  $p$ -hydroxyanizole had 41 % and 38 %, respectively, of the antioxidant activities whereas gomisin N and  $\gamma$ -schizandrin had 24 % and 22 %, respectively, of the antioxidant activities.

Example 4. Determination of melanin synthesis inhibition activity in  
10 *Streptomyces bikiniensis*

The melanin synthesis inhibition activity of gomisin N and  $\gamma$ -schizandrin obtained from Example 1 in *Streptomyces bikiniensis* was determined, and the result is shown in Table 2. When compared to  $p$ -hydroxyanizole, which has been clinically tried as a strong melanin synthesis inhibitor in skin cancer patients,  $\gamma$ -schizandrin has a somewhat weak activity at higher concentrations than 50  $\mu$ g, but showed similar activity around 20  $\mu$ g. Gomisin N had a somewhat weaker activity than  $p$ -hydroxyanizole and  $\gamma$ -schizandrin, but showed activity at as low as 12.5  $\mu$ g.

Table 2

| Compound                 | Diameter of inhibition zone (mm) |            |            |              |           |
|--------------------------|----------------------------------|------------|------------|--------------|-----------|
|                          | 10 $\mu$ g                       | 50 $\mu$ g | 25 $\mu$ g | 12.5 $\mu$ g | 6 $\mu$ g |
| <i>p</i> -hydroxyanizole | 35                               | 25         | 15         | 12           | 10        |
| Gomisin N                | 20                               | 18         | 12         | 10           | 0         |
| $\gamma$ -schizandrin    | 25                               | 20         | 15         | 12           | 10        |

#### Example 5. Aspergillin synthesis inhibition activity

In a 24 well-plate, PDA medium containing the *A.niger* spore suspension was added and solidified. Gomisin N and  $\gamma$ -schizandrin obtained from Example 1 were serially diluted from 500  $\mu$ g/ml with ethanol. The pigment synthesis of the spores was observed visually, and the result is shown in Figure 3. In this figure, the following representations are used; C: 100 % ethanol, H: *p*-hydroxyanizole, G: gomisin N, S:  $\gamma$ -schizandrin. After cultivating at 37 °C for 2 days, both gomisin N and  $\gamma$ -schizandrin showed activities at 120  $\mu$ g/ml. In the control group treated with *p*-hydroxyanizole, strong activity was observed at 60  $\mu$ g/ml. Gomisin N and  $\gamma$ -schizandrin, however, did not show antimicrobial activity against *A.niger* but only inhibited the pigment synthesis of the spores. On the other hand, the cytotoxicity against *A.niger* was observed at 500  $\mu$ g/ml and 250  $\mu$ g/ml of *p*-hydroxyanizole.

#### Example 6. Determination of melanin synthesis inhibition activity

B16 mouse melanoma cells were treated with gomisin N and  $\gamma$ -schizandrin at 10  $\mu$ g/ml and 20  $\mu$ g/ml, respectively and cultured. Cells were



centrifuged and observed visually. The results show that the activity of them even at 10  $\mu\text{g/ml}$  was stronger than that of albutin at 100  $\mu\text{g/ml}$  used as the positive control. Moreover, cytotoxicity was not observed even at 100  $\mu\text{g/ml}$  of gomisin N and  $\gamma$ -schizandrin. The result is illustrated in Figure 4 (SHC1: 5 gomisin N, SHC2:  $\gamma$ -schizandrin). Therefore, the compounds isolated from schizandra are confirmed to have a strong melanin synthesis inhibition effect without noticeable cytotoxicity.

Also,  $\text{IC}_{50}$  value was calculated by measuring the amount of the produced melanin after extracting melanin from the cells treated with gomisin N, and was 10 10  $\mu\text{g/ml}$ . Since the  $\text{IC}_{50}$  value of albutin is 300  $\mu\text{g/ml}$ , gomisin N is a very strong whitening compound that inhibits melanin synthesis.

#### Example 7. Determination of allergy induction

The 8 mm Finn chambers spread with 15 mg each of gomisin N were 15 adhered on the fore arm region of the 40 healthy male and female volunteers for 24 hours and removed. The degree of allergic reaction was observed by eye in 2 hours after detachment, and determined according to the standard set by the Cosmetic, Toiletry and Fragrance Association Inc. The degree of skin irritation was calculated by adding the values of all the examinees and dividing sum by the 20 number of the examinees.

Table 3 Standard table to determine the skin irritation by eye

| Level | Degree of reaction | Symptom                               |
|-------|--------------------|---------------------------------------|
| 0     | -                  | No reaction                           |
| 1     | +/-                | Minimal erythema                      |
| 2     | +                  | Erythema                              |
| 3     | ++                 | Erythema, inflammation                |
| 4     | +++                | Erythema, inflammation, water blister |

The allergic reaction does not occur if the degree of skin irritation is 3.0 or  
5 below. The degree of skin irritation was 2.0 for gomisin N.

The present invention provides a melanin synthesis inhibition compound that has an excellent whitening effect and low cytotoxicity. The melanin synthesis inhibition compound of the present invention can be used a cosmetic or pharmaceutical skin whitener or food coloration inhibitor.

**CLAIMS**

1. A melanin synthesis inhibition composition containing gomisin N or  $\gamma$ -schizandrin.
- 5 2. The composition according to claim 1, wherein said composition is a skin whitener.
3. The composition according to claim 2, wherein said composition is a cosmetic.
4. The composition according to claim 2, wherein said composition is a  
10 pharmaceutical skin whitener.
5. The composition according to claim 2, wherein said composition is a food coloration inhibitor.

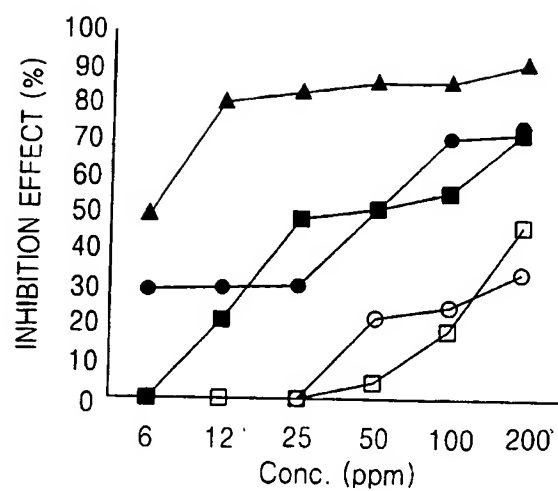
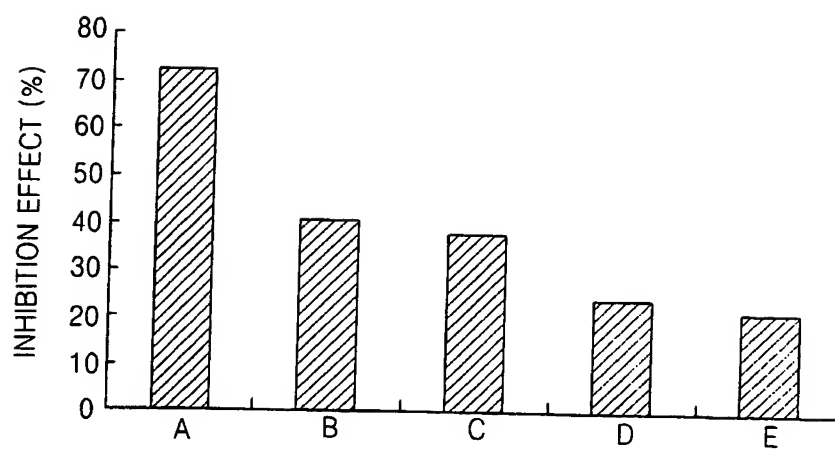
1/2  
FIG. 1

FIG. 2



2/2

FIG. 3

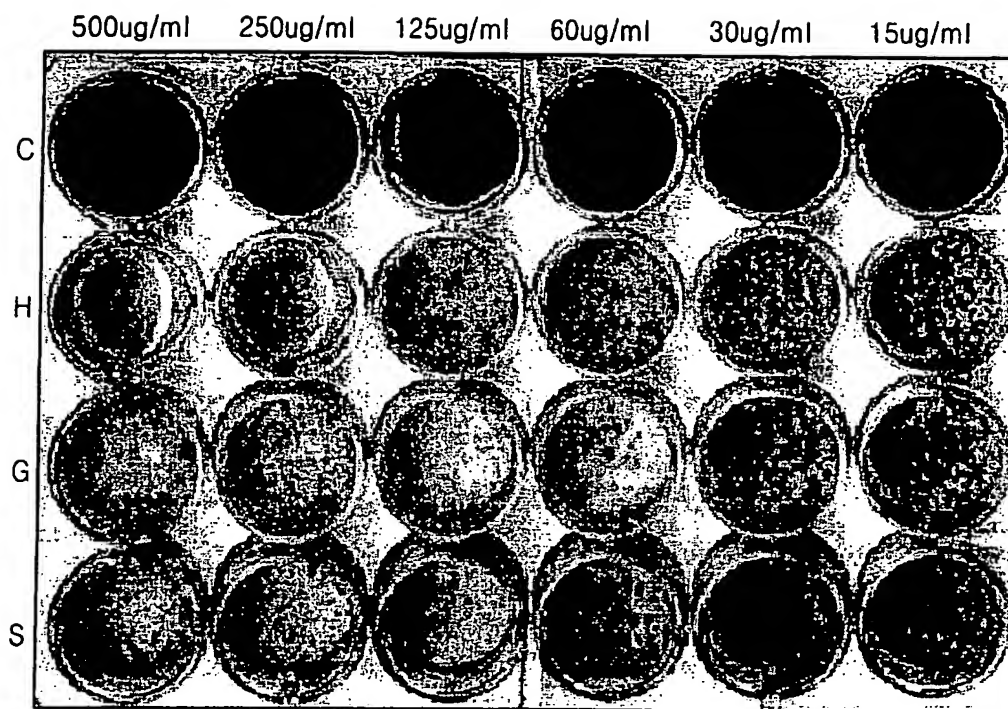
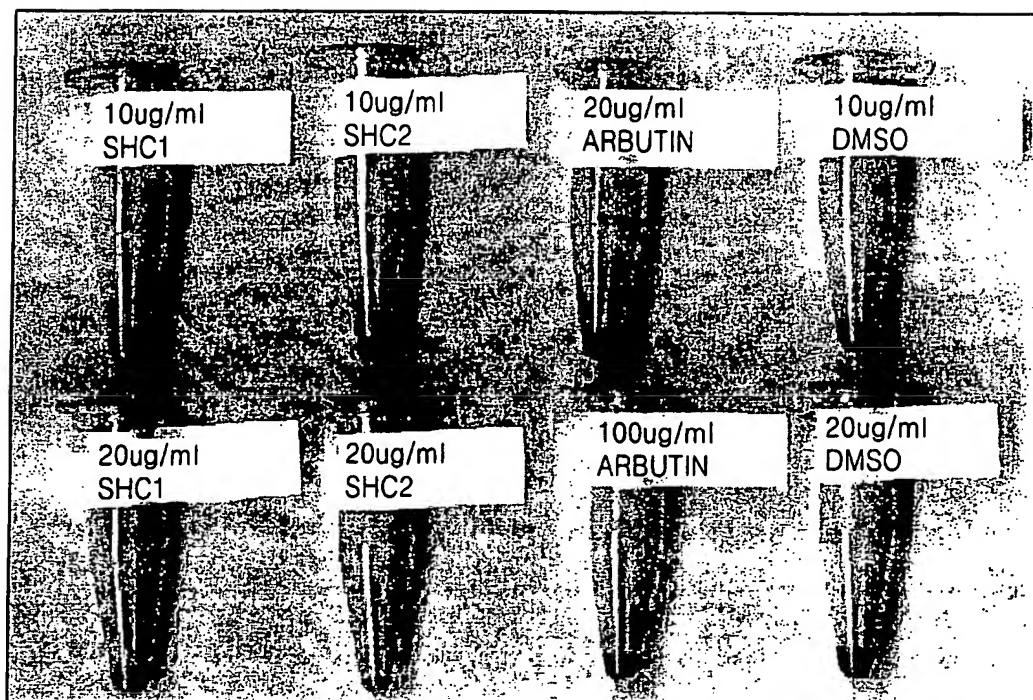


FIG. 4



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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/KR00/01420

**A. CLASSIFICATION OF SUBJECT MATTER****IPC7 A61K 35/78**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

ICP7: A61K 35/78, A61K 35/70

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and application for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, NPS, PAJ, CA on line, STN on line

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages           | Relevant to claim No. |
|-----------|--|-----------------------|
| Y         | JP 60-42485 A2 (OSAKA CHEM LAB.) 6 Mar 1985 (Family none)<br>see the whole document          | 1-5                   |
| Y         | JP 64-16721 A2 (TSUMURA & CO) 20 Jan 1989 (Family none)<br>page 1, lines 1-20                | 1-5                   |
| A         | GANGUANG KEXUE YU GUANG HUAXUE, vol. 15, no. 2, pp. 114-119 (1997)<br>see the whole document | 1-3                   |
| A         | PLANTA MED., vol. 58, no. 4, pp. 311-313 (1992)<br>see the whole document                    | 1-3                   |

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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Date of the actual completion of the international search

26 MARCH 2001 (26.03.2001)

Date of mailing of the international search report

28 MARCH 2001 (28.03.2001)

Name and mailing address of the ISA/KR

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